Detection of *Candida* species resistant to azoles in the microbiota of rheas (*Rhea americana*): possible implications for human and animal health

Raimunda Sâmia Nogueira Brilhante,¹ Lucas Pereira de Alencar,^{1,2}
Rossana de Aguiar Cordeiro,¹
Débora de Souza Collares Maia Castelo-Branco,¹
Carlos Eduardo Cordeiro Teixeira,¹ Ramila de Brito Macedo,¹
Daniel Teixeira Lima,¹ Manoel de Araújo Neto Paiva,^{1,2}
André Jalles Monteiro,³ Nilza Dutra Alves,⁴ Moacir Franco de Oliveira,⁴
José Júlio Costa Sidrim,¹ Marcos Fábio Gadelha Rocha,^{1,2} Tereza de Jesus
Pinheiro Gomes Bandeira^{1,5} and Terezinha de Jesus Santos Rodrigues¹

Correspondence

Raimunda Sâmia Nogueira
Brilhante
brilhante@ufc.br

¹Department of Pathology and Legal Medicine, School of Medicine, Specialized Medical Mycology Center, Postgraduate Program in Medical Microbiology, Federal University of Ceará, Fortaleza, CE, Brazil

²School of Veterinary, Postgraduate Program in Veterinary Science, State University of Ceará, Fortaleza, CE, Brazil

³Department of Statistics and Applied Mathematics, Federal University of Ceará, Fortaleza, CE, Brazil

⁴Department of Animal Science, Federal Rural University of the Semiárid, Mossoró, RN, Brazil

⁵School of Medicine, Christus College, UNICHRISTUS, Fortaleza, CE, Brazil

There is growing interest in breeding rheas (*Rhea americana*) in Brazil. However, there are no data on the yeast microbiota of the gastrointestinal tract of this avian species, and the phenotypic characteristics of these yeasts are not known. Therefore, the aim of this work was to isolate *Candida* species from the digestive tract of rheas and to evaluate the *in vitro* antifungal susceptibility and secretion of phospholipases of the recovered isolates. For this purpose, 58 rheas from breeding operations in the cities of Fortaleza and Mossoró, north-eastern Brazil, were used. Samples were gathered from the oropharynx and cloaca of the animals using sterile swabs. Stool samples were collected from their pens by scraping with a scalpel blade. For the primary isolation, the material was seeded onto 2 % Sabouraud dextrose agar supplemented with chloramphenicol (0.5 g l⁻¹). The isolates were identified based on morphological and biochemical features. After identification, all the strains were submitted to antifungal susceptibility testing for amphotericin B, itraconazole and fluconazole. The phospholipase activity of the *Candida* species isolates was also tested by culturing on egg yolk agar. *Candida* species were isolated from at least one anatomical site in 36/58 birds (14/17 juveniles and 22/41 adults) and in 6/10 faecal samples. Mostly, only a single species was isolated from each collection site (36/56 positive sites), with up to three species being observed only in four cases (4/56). A total of 77 isolates were obtained, belonging to the species *Candida parapsilosis sensu lato* (19), *Candida albicans* (18), *Candida tropicalis* (13), *Candida guilliermondii* (12), *Candida krusei* (10) and *Candida famata* (5). *C. albicans* was more prevalent in the oropharynx of the juvenile rheas when compared with adult ones ($P < 0.001$). All tested isolates were susceptible to amphotericin B, but 16 isolates were simultaneously resistant to the two azole derivatives (11/18 *C. albicans*, 1/10 *C. krusei*, 2/19 *C. parapsilosis sensu lato* and 2/13 *C. tropicalis*). *C. albicans* presented a particularly high resistance rate to fluconazole (15/18) and itraconazole (13/18). Finally, 23/77 strains secreted phospholipases. In summary, healthy rheas carry potentially pathogenic *Candida* species in their gastrointestinal tract, including azole-resistant strains that secrete phospholipases, and are prone to disseminating them in the environment. Thus, breeding and handling these animals may have some implications for human and animal health.

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INTRODUCTION

The greater rhea (*Rhea americana*) is a large flightless bird with a close phylogenetic relationship with the ostrich (*Struthio camelus*), cassowary (*Casuarius* sp.), emu (*Dromaius novaehollandiae*) and kiwi (*Apteryx australis*). The species is native to South America and is found in the wild in many regions of Brazil, despite threats from hunting and habitat destruction (Parizzi *et al.*, 2007; Soares *et al.*, 2010; IUCN, 2012). Rheas are omnivorous, feeding on many plant species, small rodents, reptiles and insects. In recent years, there has been an increase in the number of rhea farms in Brazil to produce meat and feathers.

There are very few studies on the sanitary aspects of commercial breeding of rheas. There is no information on the micro-organisms present in these birds and whether contact with these animals facilitates the dissemination of infectious agents to new hosts and environments. Rheas, as other wild animals, possibly increase the dispersion of ecological niches in the transmission chain of diseases, both to humans and to other animals (Azevedo *et al.*, 2010; Soares *et al.*, 2010). Therefore, it is necessary to investigate animals that are widely dispersed in the wild and also bred in captivity for their possible role as reservoirs of pathogenic micro-organisms, even in the absence of clinical signs of infection.

Yeasts are micro-organisms commonly found in the gastrointestinal microbiota of birds (Mancianti *et al.*, 2002; Melville *et al.*, 2004; Cafarchia *et al.*, 2006a; Brilhante *et al.*, 2010; Costa *et al.*, 2010). There are reports of antifungal resistance among *Candida* species isolated from healthy cockatiels (Sidrim *et al.*, 2010). The microbiota can vary according to the host species (Melville *et al.*, 2004; Cafarchia *et al.*, 2006b; Brito *et al.*, 2009; Brilhante *et al.*, 2010), but generally there is a high frequency of isolation of *Candida* species, particularly *Candida albicans*. To the best of our knowledge, however, there have been no specific studies on the microbiota of *Candida* species of rheas or the antifungal resistance and virulence of the micro-organisms found. Therefore, the objective of this work was to isolate yeasts of the genus *Candida* in the microbiota of these birds and to characterize these isolates for resistance to antifungal agents in clinical use and to analyse their production of phospholipases, an important virulence factor commonly detected in yeasts isolated from birds.

METHODS

Animals. Fifty-eight apparently healthy greater rheas (*R. americana*) of both sexes, bred in captivity in the cities of Fortaleza, Ceará (3°43'6" S, 38°32'36" W) and Mossoró, Rio Grande do Norte (5°11'17" S, 37°20'39" W), north-eastern Brazil, were studied, of which 17 were juveniles (≤ 12 months) and 41 adults (> 12 months). The birds were physically restrained, and before specimen collection, they were individually evaluated. This study was approved by the Animal Research Ethics Committee of Ceará State University (protocol no. 11044670-4/43).

Sample collection. Samples were collected from two anatomical sites (oropharynx and cloaca) and from droppings. Sterile cotton swabs were used to obtain samples from the oropharynx and cloaca. The swabs were inserted into the anatomical site and rotated. They were then placed into sterile glass slants, containing 1 ml sterile saline (0.9% NaCl), keeping the cotton extremity in contact with the solution at 10 °C until processing, within 12 h. A total of ten stool samples composed of a pool of faeces were collected from the pens where the birds were maintained and were kept at 10 °C in sterile saline solution until processing, within 12 h.

Isolation and fungal identification procedures. The swabs with samples from the oropharynx and cloaca were inoculated in Petri dishes containing 2% Sabouraud dextrose agar (Difco Laboratories) with chloramphenicol and incubated at 25 °C for 10 days, during which the dishes were observed daily for evaluation of the presence of fungal colonies. Previous to streaking the agar plates, the stool samples were processed as described by Brilhante *et al.* (2010). The cultured media were incubated at 25 °C for 10 days. For each positive sample, colonies were observed microscopically after Gram staining. For *Candida* species, the colonies were initially grown on chromogenic medium (HiChrome *Candida* Differential Agar; HiMedia) for identification of mixed colonies. Afterwards, individual colonies were subcultured onto slants containing potato dextrose agar and Christensen's urea agar, and after 24–48 h, the micro-organisms were grown on cornmeal/Tween 80 agar for morphological analysis. A sugar assimilation test was also performed for each isolate and the results were interpreted according to De Hoog *et al.* (2000).

Antifungal susceptibility testing. *Candida* strains were tested against amphotericin B (AMB; Sigma), fluconazole (FLC; Pfizer) and itraconazole (ITC; Janssen Pharmaceutica). The antifungal drugs were diluted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2008). The concentration range tested was 0.03125–16 µg ml⁻¹ for AMB and ITC and 0.125–64 µg ml⁻¹ for FLC (Brito *et al.*, 2009; Sidrim *et al.*, 2010). Inocula were prepared from 2-day-old cultures grown on potato dextrose agar at 35 °C to reach a final concentration of 0.5×10^3 – 2.5×10^3 cells ml⁻¹ in RPMI buffered to pH 7 with 0.165 M MOPS, as recommended by CLSI (2008). Susceptibility was investigated by broth microdilution in 96-well microdilution plates at 35 °C for 48 h. All isolates were tested in duplicate. For AMB, MIC was defined as the lowest concentration at which no growth was observed; for ITC and FLC, MICs were defined as the lowest drug concentration inhibiting fungal growth by 50% when compared with the control well (CLSI, 2008). Isolates with MICs > 1 , ≥ 64 and ≥ 1 µg ml⁻¹ were considered resistant to AMB, FLC and ITC, respectively (CLSI, 2008), except for *C. albicans*, *Candida parapsilosis sensu lato* and *Candida tropicalis* against FLC, which were considered resistant at MIC ≥ 8 (Pfaller, 2012). *C. parapsilosis* ATCC 22019 was included as a quality control for each test (CLSI, 2008).

Phospholipase experiments. All strains were tested for phospholipase activity (Pz). The test was performed according to Sidrim *et al.* (2010). Briefly, the medium used was 2% Sabouraud dextrose agar, supplemented with 1 mol sodium chloride l⁻¹, 0.05 mol calcium chloride l⁻¹ and 8% sterile egg yolk emulsion, at a concentration of 30%. The emulsion was heated to 40 °C and incorporated into the sterile Sabouraud medium after it reached the same temperature. The medium was poured into 90 mm Petri dishes and the yeast inoculum was prepared in sterile saline at a concentration of 4 on the McFarland standard scale. The inoculum (5 µl) was dripped onto a 5 mm sterilized filter paper disc, which was then placed on the agar. The plates were then incubated at 35 °C for 7 days. Pz was determined by calculating the ratio between the diameter of the yeast colony and the total diameter comprising the colony and the precipitation zone. According to this criterion, when Pz=1, the

isolate is phospholipase negative; when $1 > P_z \geq 0.64$ the isolate is positive for Pz; and when $P_z < 0.64$, the isolate is strongly positive for this enzyme.

Statistical analysis. Fisher's exact test was used to check for differences in the number of isolates between the anatomical sites and bird age ranges (adult versus juvenile). Student's *t*-test for paired samples was used to compare the MICs in relation to the antifungal drugs, as well as for comparisons between the sites in relation to the species isolated. To compare the species irrespective of collection site, analysis of variance was used with *post hoc* application of Fisher's test. Analysis of variance was also employed to assess differences in the production of phospholipases, both among species and between their isolation sites. Results with $P < 0.05$ were considered significant.

RESULTS

A total of 36/58 rheas were positive for growth of *Candida* species in at least one of the sites sampled (14/17 for juveniles and 22/41 for adult birds). The positivity per site was 29/58 in the oropharynx and 21/58 in the cloaca. In 14/58 animals, *Candida* species were recovered from both anatomical sites. Six of the ten stool samples were positive.

Only one *Candida* species was isolated from 39/56 positive samples, whilst in 13/56 cases two species were isolated and in 4/56 cases, three species were isolated (Table 1). Therefore, a total of 77 *Candida* isolates were obtained, comprising six species, as described in Table 2. The most frequently isolated species were *C. parapsilosis sensu lato* (19/77) and *C. albicans* (18/77). *C. albicans* was significantly more frequent in the oropharynx of juvenile birds than in adults ($P < 0.0001$) (Table 2).

The results obtained from antifungal susceptibility testing are shown in Table 3. There was no resistance to AMB among the isolates. However, 16 strains were resistant to both azole derivatives, namely *C. albicans* (11/18), *Candida*

krusei (1/10), *C. parapsilosis sensu lato* (2/19) and *C. tropicalis* (2/13). There was a significant occurrence of resistance among the strains of *C. albicans* to FLC (15/18) and ITC (13/18). Other isolates showed resistance as follows: *C. parapsilosis sensu lato*, 7/19 and 3/19 to FLC and ITC, respectively; *C. tropicalis*, 6/13 and 5/13 to FLC and ITC, respectively; *Candida guilliermondii*, 1/12 to ITC; and *C. krusei*, 2/10 and 1/10 to FLC and ITC, respectively.

The P_z of the 77 strains of *Candida* species tested is shown in Table 4. Fifty-four strains were negative ($P_z = 1$) for the excretion of phospholipase, whilst 18 were positive ($1 > P_z \geq 0.64$) and five were strongly positive ($P_z < 0.64$). The mean P_z of the strains that were positive was 0.75, for 9/18 *C. albicans*, 1/5 *Candida famata*, 2/12 *C. guilliermondii*, 4/19 *C. parapsilosis sensu lato* and 2/13 *C. tropicalis*. The mean P_z in strongly positive strains was 0.60, for 1/12 *C. guilliermondii*, 3/19 *C. parapsilosis sensu lato* and 1/13 *C. tropicalis*. No significant difference was found concerning the secretion of phospholipases by *Candida* species.

DISCUSSION

We believe that this is the first study to focus on *Candida* species isolated from the oropharynx, cloaca and droppings of rheas with the purpose of analysing the occurrence of resistance to antifungals in healthy birds. There have been reports of invasive pulmonary fungal infection in rheas caused by *Aspergillus fumigatus* (Reissig *et al.*, 2002; Copetti *et al.*, 2004), but there are no data on *Candida* colonization in healthy rheas.

A high recovery rate of *Candida* species was observed (62.07%), which was much higher than the isolation of yeasts in birds of prey in Europe (25%; Cafarchia *et al.*, 2006a), similar to that of cockatiels (65%; Brilhante *et al.*,

Table 1. Number (%) of positive samples from oropharynx, cloaca and faeces, grouped according to the species of yeast isolated

<i>Candida</i> species	Oropharynx	Cloaca	Faeces	Total
<i>C. albicans</i>	11 (37.9)	3 (14.3)	–	14
<i>C. famata</i>	2 (7.0)	–	2 (33.3)	4
<i>C. guilliermondii</i>	3 (10.3)	1 (4.8)	–	4
<i>C. krusei</i>	2 (7.0)	2 (9.5)	–	4
<i>C. parapsilosis sensu lato</i>	2 (7.0)	3 (14.3)	2 (33.3)	7
<i>C. tropicalis</i>	1 (3.4)	5 (23.7)	–	6
<i>C. albicans</i> + <i>C. famata</i>	1 (3.4)	–	–	1
<i>C. albicans</i> + <i>C. guilliermondii</i>	–	1 (4.8)	–	1
<i>C. albicans</i> + <i>C. krusei</i>	1 (3.4)	–	–	1
<i>C. albicans</i> + <i>C. parapsilosis</i>	–	1 (4.8)	–	1
<i>C. guilliermondii</i> + <i>C. krusei</i>	1 (3.4)	–	–	1
<i>C. guilliermondii</i> + <i>C. krusei</i> + <i>C. parapsilosis sensu lato</i>	–	1 (4.8)	1 (16.7)	2
<i>C. guilliermondii</i> + <i>C. krusei</i> + <i>C. tropicalis</i>	1 (3.4)	–	–	1
<i>C. guilliermondii</i> + <i>C. parapsilosis sensu lato</i>	2 (7.0)	–	1 (16.7)	3
<i>C. krusei</i> + <i>C. parapsilosis sensu lato</i> + <i>C. tropicalis</i>	1 (3.4)	–	–	1
<i>C. parapsilosis sensu lato</i> + <i>C. tropicalis</i>	1 (3.4)	4 (19)	–	5
Total	29 (100)	21 (100)	6 (100)	56

Table 2. Frequency of *Candida* species according to collection site and animal age

<i>Candida</i> species	Oropharynx		Cloaca		Faeces	Total
	Juvenile	Adult	Juvenile	Adult		
<i>C. albicans</i>	11*	2*	3	2	–	18
<i>C. famata</i>	1	2	–	–	2	5
<i>C. guilliermondii</i>	2	5	–	3	2	12
<i>C. krusei</i>	2	4	–	3	1	10
<i>C. parapsilosis sensu lato</i>	2	4	2	7	4	19
<i>C. tropicalis</i>	2	2	4	5	–	13
Total	20	19	9	20	9	77

*Statistically significant difference between animal ages ($P<0.05$).

2010) and lower than that of Brazilian raptors (72.73 %; Brilhante *et al.* 2012). The isolation rate of *Candida* species in the oropharynx of the rheas was higher than that observed in ostriches and cockatiels (Melville *et al.*, 2004; Brilhante *et al.*, 2010), and the isolation in the cloaca was higher than in ostriches and migratory birds (Melville *et al.*, 2004; Cafarchia *et al.*, 2006b). Additionally, it was observed that rhea droppings provide a good substrate for colonization by *Candida* species, as shown by the recovery of this yeast genus from 6/10 (60 %) samples, greater than the recovery rate observed in studies with other bird species (Mancianti *et al.*, 2002; Cafarchia *et al.*, 2006a; Brilhante

et al., 2010). This finding suggests that care should be taken when handling these birds and their excrements.

These differences between the recovery rates of *Candida* species from different anatomical sites of several avian species are probably associated with species-specific factors, such as the ecology, diet and habitat of these animals (Cafarchia *et al.*, 2006b). In contrast, despite influencing the recovery rate of *Candida* from different collection sites, these species-specific factors do not seem to influence the composition of the gastrointestinal yeast microbiota, as the species of *Candida* recovered in this study were similar to other reports concerning other avian groups, such as

Table 3. *In vitro* antifungal susceptibility of *Candida* species isolated from the gastrointestinal tract of rheas

GM, Geometric mean; S, sensitive; R, resistant; –, not detected; NC, not calculated because of the small number of strains, which did not allow reliable results.

<i>Candida</i> species (n)	Drug	Range	MIC ($\mu\text{g ml}^{-1}$)			Classification (n)	
			GM	MIC ₅₀	MIC ₉₀	S	R
<i>C. albicans</i> (18)	AMB	0.0625–1	0.25	0.25	0.55	18	–
	FLC	1–>64	33.26	64.00	128.0	3	15
	ITC	0.0625–>16	5.879	16.00	32.00	5	13
<i>C. famata</i> (5)	AMB	0.03125–0.5	0.125	NC	NC	5	–
	FLC	0.25 –16	3.48	NC	NC	5	–
	ITC	0.0625–0.5	0.143	NC	NC	5	–
<i>C. guilliermondii</i> (12)	AMB	0.03125–0.5	0.118	0.187	0.425	12	–
	FLC	0.5–32	3.175	4.0	27.20	12	–
	ITC	0.03125–>16	0.166	0.125	22.55	11	1
<i>C. krusei</i> (10)	AMB	0.0625–1	0.25	0.25	0.95	10	–
	FLC	0.125–>64	4.287	2.00	121.6	8	2
	ITC	0.0625–>16	0.267	0.187	28.85	9	1
<i>C. parapsilosis sensu lato</i> (19)	AMB	0.03125–0.5	0.12	0.125	0.25	19	–
	FLC	0.125–>64	2.489	4.0	32.0	12	7
	ITC	0.03125–>16	0.268	0.125	32.0	16	3
<i>C. tropicalis</i> (13)	AMB	0.03125–1	0.146	0.125	0.8	13	–
	FLC	0.125–32	3.232	4.0	32.0	7	6
	ITC	0.0625–>16	1.0	0.25	32.00	8	5

Table 4. Pz of the *Candida* species strains

<i>Candida</i> species	<i>n</i>	Pz [mean $\pm$ SD (<i>n</i>)]		No. negative isolates (Pz=1)
		Positive isolates ($1 > Pz \geq 0.64$)	Strongly positive isolates ($Pz < 0.64$)	
<i>C. albicans</i>	18	0.74 $\pm$ 0.09 (9)	–	9
<i>C. famata</i>	5	0.65 (1)	–	4
<i>C. guilliermondii</i>	12	0.77 $\pm$ 0.14 (2)	0.55 (1)	9
<i>C. krusei</i>	10	–	–	10
<i>C. parapsilosis sensu lato</i>	19	0.74 $\pm$ 0.06 (4)	0.62 $\pm$ 0.01 (3)	12
<i>C. tropicalis</i>	13	0.85 $\pm$ 0.07 (2)	0.61 (1)	10
Total	77	0.75 $\pm$ 0.09 (18)	0.60 $\pm$ 0.03 (5)	54

ostriches (Melville *et al.*, 2004), psittacines (Vieira & Coutinho, 2009; Brilhante *et al.*, 2010) and raptors (Brilhante *et al.*, 2012).

C. parapsilosis sensu lato was the most prevalent species in this study, occurring in both anatomical sites of young and adult animals. This yeast is an emerging pathogen that has become more prevalent in humans and animals in recent years. It is the non-*C. albicans* species that has been most often reported in cases of invasive candidiasis, mainly in immunocompromised individuals (Trofa *et al.*, 2008; Lord *et al.*, 2010). In recent studies, it was shown that, of 141 bloodstream isolates tested in Brazil, 88% were *C. parapsilosis*, 9% *Candida orthopsilosis* and 3% *Candida metapsilosis* (Gonçalves *et al.*, 2010), whilst in Spain, of 293 isolates recovered from cases of invasive candidiasis, 74.4% were *C. parapsilosis*, 23.54% were *C. orthopsilosis* and 2.05% were *C. metapsilosis* (Garcia-Effron *et al.*, 2012). However, the distribution of strains from veterinary sources among the three species of the *C. parapsilosis* species complex is still unknown. Thus, additional studies will be performed in order to further identify these strains.

C. albicans was the second most frequent species in this study and has been reported as the most common *Candida* sp. among avian hosts (Garner *et al.*, 2010), being widely recovered from several different bird species, such as ostriches (Melville *et al.*, 2004), cockatiels (Brilhante *et al.*, 2010) and raptors (Brilhante *et al.*, 2012). A greater frequency of *C. albicans* in the oropharynx of birds, as observed here, has also been reported by other authors (Melville *et al.*, 2004; Brilhante *et al.*, 2010), suggesting that this yeast is one of the main components of the microbiota of this anatomical site in avian species. In humans, *C. albicans* is often isolated in both healthy and sick hosts and is the most common species in infections, accounting for over half of invasive candidiasis cases (Lass-Flörl, 2009; Lord *et al.*, 2010).

Other non-*C. albicans* species have increasingly been reported in human and animal infections, sometimes being classified as emerging pathogens (Deem, 2003; Desnos-Ollivier *et al.*, 2008; Lord *et al.*, 2010). Several of these species were isolated in this study, such as *C. famata*, *C. guilliermondii*, *C. tropicalis* and *C. krusei*. *C. famata* is

considered to be a non-pathogenic commensal yeast, but has been reported in infections of the ocular system and central nervous system and in cases of candidaemia (Nawange *et al.*, 2010). *C. guilliermondii* has been reported in superficial infections, such as onychomycosis, and, rarely, in invasive infections, including bone infections and candidaemia (Pfaller *et al.*, 2006). *C. tropicalis* is among the most mentioned non-*C. albicans* species involved in infections (Ortega *et al.*, 2011), and can be isolated from cancer patients, intensive care units and post-operative patients. It caused the highest mortality in cases of candidaemia, when compared with the other *Candida* species in a study carried out in Italy (Tortorano *et al.*, 2002; Ortega *et al.*, 2011). *C. krusei* is involved in around 4% of infections by members of the genus *Candida*, being isolated more often from patients with blood disorders, from transplant patients and from patients who have previously been exposed to azole-based antifungals (Ortega *et al.*, 2011).

Predominantly, collection sites were colonized by one single species (69.64% of positive samples). In turn, two *Candida* species were found in 23.21% of the samples and three were found in 7.14%. The same pattern has been observed in other bird species (Melville *et al.*, 2004; Cafarchia *et al.*, 2006a; Vieira & Coutinho, 2009; Brilhante *et al.*, 2010). The predominance of a single yeast species in most collection sites suggests that some species are able to inhibit colonization by others and, as demonstrated by Brilhante *et al.* (2010), *C. albicans* was most commonly found as the only species in a given collection site. This ability to inhibit the growth of other species may contribute to the overgrowth of a certain strain, as microbial competition will be reduced, leading to the development of a *Candida* infection, especially when predisposing factors are present. This mechanism is probably involved in the capacity of *C. albicans* to be particularly pathogenic in immunocompromised hosts (Rodríguez-Galán *et al.*, 2010). Considering the high recovery rate of *Candida* species, breeders should be aware of changes in the parasite–host balance, as some factors such as stress, poor sanitation, overcrowding and poor nutrition among confined animals favour the multiplication of these micro-organisms and facilitate their

dissemination from the colonized sites, leading to the development of infection (Vieira & Coutinho, 2009; Rodríguez-Galán *et al.*, 2010).

Regarding the *in vitro* antifungal susceptibility, no isolates were resistant to amphotericin B, showing that the strains recovered from rheas react to this drug similarly to those isolated from other healthy animals (Brito *et al.*, 2009; Sidrim *et al.*, 2010; Brilhante *et al.*, 2011, 2012).

In contrast, a substantial resistance to azole derivatives was observed among the *Candida* species assessed in the present study. Isolates of *C. albicans*, *C. krusei*, *C. parapsilosis sensu lato* and *C. tropicalis* presented resistance to both azole drugs tested. This finding is particularly important because all of these species have been implicated as human or animal pathogens (Deem, 2003; Lord *et al.*, 2010; Sidrim *et al.*, 2010). Additionally, resistance to ITC in isolates of *C. guilliermondii* was also observed. The high resistance rate to azole derivatives among *Candida* species in this study corroborates the results of other studies with *Candida* species from healthy animals and environmental sources (Brito *et al.*, 2009; Lord *et al.*, 2010; Sidrim *et al.*, 2010; Brilhante *et al.*, 2011, 2012).

Resistance to azole derivatives is fundamentally linked to changes in gene sequence or expression and, as a rule, appears to be due to selective pressures induced by exposure to the azole products used in clinical practice or in agriculture (Kanafani & Perfect, 2008; Brilhante *et al.*, 2012; Pfaller, 2012). As the animals evaluated in this study had no history of prior treatment with antifungal drugs, the resistance may be associated with the presence of these compounds in the feed or fruits given to these animals, as the occurrence of cross-resistance to medical and agricultural azoles in *Candida* species has already been reported (Müller *et al.*, 2007).

The mean Pz activity in the *Candida* species of this study was lower than observed in studies with *Candida* species from other avian species (Cafarchia *et al.*, 2008; Vieira & Coutinho, 2009; Sidrim *et al.*, 2010) and from healthy humans (Oksuz *et al.*, 2007). Some authors have observed a correlation between resistance to FLC and secretion of phospholipases (Ying & Chunyang, 2012). This might have occurred in our study, where >80% of the *C. albicans* strains were resistant to FLC and 50% were positive for production of this enzyme. Non-*C. albicans* species in a smaller proportion also secreted phospholipases, including strains of *C. guilliermondii*, *C. parapsilosis sensu lato* and *C. tropicalis*.

Even though *Candida* species are considered commensal organisms and compose the microbiota of the gastrointestinal tract of birds, they are capable of causing disease whenever an impairment of the immune system is established. In recent years, the incidence of opportunistic mycoses, especially those caused by *Candida* species, has increased (Pfaller & Diekema, 2007). In addition, it has been suggested that animals should be considered potential

sources of *Candida* species infections for humans, mainly immunocompromised individuals (Edelmann *et al.*, 2005). Thus, based on these assumptions, the recovery of resistant *Candida* isolates capable of producing phospholipase from the gastrointestinal tract is important with regard to the possible transmission of resistant strains from these animals to humans.

CONCLUSION

Healthy rheas (*R. americana*) carry potentially pathogenic *Candida* species in their gastrointestinal tract, including azole-resistant strains that secrete phospholipases, and are prone to disseminating them in the environment. Thus, breeding and handling of these animals may have some implications for human and animal health.

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